THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH
66TH STREET AND YORK AVENUE
NEW YORK 21, N.Y.

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Dear Joshua :

While I try to make some sense out of those so old and long neglected notes let me make a few suggestions on another tack. They may not be pertinent as I'm not sure exactly what you want. If it is to obtain a lysate from a TM2 culture that has been transduced for a particular marker and has become concommittantly lysogenized I suggest that you obtain this particular transduction using the H4 phage that I sent you as it does not stably lysogenize the transductions and can be purified away by streaking.

It is not that TM2(P22) doesn't induce very well but rather that the induced infective center is more sensitive to UV than free phage. You can get up to 50% induction under the following conditions. Grow cells in broth to ca, 2.10^8/ ml* (log phage) and wash thoroughly in buffer. Irradiate in a thin layer with shaking for ten seconds at 53 cm from your UV germicidal lamp then resuspend in 2X broth. Incubate for two hours with aeration, shake out thoroughly with chloroform and sediment the debris. This should give about 10^9 phage per ml. The difficulty is that the unlysed fraction of cells grow out and adsorb the newly produced phage but these should be enough left for use. If necessary concentrate in the Spinco or its equivalent using some high titer T2 as carrier . The T2 will have no effect on the assay. One can also suspend cells (to be transduced) at 5.10^7/ ml(log phase) in dilute phage* (up to 100ml is convenient) an let them adsorb it out , then concentrate the cells and put on the selective medium.

I hope some of this is helpful. In the meantime I'll try to get you the rest of the information though I believe induction is your best bet if h4 won't do.

See you at Detroit.

Sincerely,

Norton

* I'm also at 10 fold dilution